



## **Patients with Long QT Syndrome Due to Impaired hERG-encoded Kv11.1 Potassium Channel Have Exaggerated Endocrine Pancreatic and Incretin Function Associated with Reactive Hypoglycemia**

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# Patients With Long-QT Syndrome Caused by Impaired *hERG*-Encoded K<sub>v</sub>11.1 Potassium Channel Have Exaggerated Endocrine Pancreatic and Incretin Function Associated With Reactive Hypoglycemia

**BACKGROUND:** Loss-of-function mutations in *hERG* (encoding the K<sub>v</sub>11.1 voltage-gated potassium channel) cause long-QT syndrome type 2 (LQT2) because of prolonged cardiac repolarization. However, K<sub>v</sub>11.1 is also present in pancreatic  $\alpha$  and  $\beta$  cells and intestinal L and K cells, secreting glucagon, insulin, and the incretins glucagon-like peptide-1 (GLP-1) and GIP (glucose-dependent insulinotropic polypeptide), respectively. These hormones are crucial for glucose regulation, and long-QT syndrome may cause disturbed glucose regulation. We measured secretion of these hormones and cardiac repolarization in response to glucose ingestion in LQT2 patients with functional mutations in *hERG* and matched healthy participants, testing the hypothesis that LQT2 patients have increased incretin and  $\beta$ -cell function and decreased  $\alpha$ -cell function, and thus lower glucose levels.

**METHODS:** Eleven patients with LQT2 and 22 sex-, age-, and body mass index-matched control participants underwent a 6-hour 75-g oral glucose tolerance test with ECG recording and blood sampling for measurements of glucose, insulin, C-peptide, glucagon, GLP-1, and GIP.

**RESULTS:** In comparison with matched control participants, LQT2 patients had 56% to 78% increased serum insulin, serum C-peptide, plasma GLP-1, and plasma GIP responses ( $P=0.03$ – $0.001$ ) and decreased plasma glucose levels after glucose ingestion ( $P=0.02$ ) with more symptoms of hypoglycemia ( $P=0.04$ ). Sixty-three percent of LQT2 patients developed hypoglycemic plasma glucose levels ( $<70$  mg/dL) versus 36% control participants ( $P=0.16$ ), and 18% patients developed serious hypoglycemia ( $<50$  mg/dL) versus none of the controls. LQT2 patients had defective glucagon responses to low glucose,  $P=0.008$ .  $\beta$ -Cell function (Insulin Secretion Sensitivity Index-2) was 2-fold higher in LQT2 patients than in controls (4398 [95% confidence interval, 2259–8562] versus 2156 [1961–3201],  $P=0.03$ ). Pharmacological K<sub>v</sub>11.1 blockade (dofetilide) in rats had similar effect, and small interfering RNA inhibition of *hERG* in  $\beta$  and L cells increased insulin and GLP-1 secretion up to 50%. Glucose ingestion caused cardiac repolarization disturbances with increased QTc intervals in both patients and controls, but with a 122% greater increase in QTcF interval in LQT2 patients ( $P=0.004$ ).

**CONCLUSIONS:** Besides a prolonged cardiac repolarization phase, LQT2 patients display increased GLP-1, GIP, and insulin secretion and defective glucagon secretion, causing decreased plasma glucose and thus increased risk of hypoglycemia. Furthermore, glucose ingestion increased QT interval and aggravated the cardiac repolarization disturbances in LQT2 patients.

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**Key Words:** arrhythmias, cardiac ■ glucagon ■ glucagon-like peptide 1 (GLP-1) ■ glucose ■ hyperglycemia ■ hypoglycemia ■ insulin-secreting cells ■ long-QT syndrome ■ potassium channels, voltage-gated

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## Clinical Perspective

### What Is New?

- Patients with long-QT syndrome type 2 (LQT2) with loss-of-function mutations in *hERG* (Kv11.1) display exaggerated incretin and endocrine pancreatic function with >50% increased levels of circulating insulin, glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide, and defective glucagon secretion, causing low plasma glucose levels, and thus increased risk of symptomatic reactive hypoglycemia following glucose ingestion.
- Pharmacological Kv11.1 blockade (dofetilide) in rats had similar effects and inhibition of *hERG* in  $\beta$  and L cells increased insulin and glucagon-like peptide-1 secretion with up to 50%.
- Furthermore, glucose ingestion aggravated cardiac repolarization disturbances in LQT2 patients with a 122% greater increase in QTcF interval and prolonged the cardiac repolarization phase in healthy controls.

### What Are the Clinical Implications?

- Glucose ingestion (75 g, equivalent of 0.6 L soft drink) led to symptomatic reactive hypoglycemia in 63% of LQT2 patients with 18% experiencing serious hypoglycemia (<50 mg/dL glucose).
- Serious hypoglycemia has previously been observed in children with LQT2, long-QT syndrome type 1, and with Kv11.1 blocking drugs.
- Hypoglycemia leads to increased propensity for QT prolongation; hypoglycemia may therefore further increase the risk of malignant arrhythmia in patients with long-QT syndrome.
- Our demonstration of LQT2 patients having increased risk of hypoglycemia should lead to a greater awareness of this risk.
- The risk of hypoglycemia can be lessened by reducing intake of easily digestible carbohydrates.

Voltage-gated potassium ( $K_v$ ) channels are known for their relation to malignant cardiac arrhythmias, where blocked or nonfunctional  $K_v$  channels cause long-QT syndrome (LQTS) because of impaired cardiac repolarization. Several inheritable mutations and many common drugs impair the function of  $K_v$  channels.<sup>1–5</sup> LQTS attributable to inherited mutations affects up to 1:2000 people. It is characterized by a prolonged QT interval and increases the risk of ventricular tachycardia of the Torsades de Pointes type, syncope, and sudden death.<sup>3,4</sup> Mutations in *KCNQ1* cause LQTS type 1 (LQT1) attributable to impaired  $K_v7.1$  channel function. Mutations in *hERG* (also known as *KCNH2*), cause LQTS type 2 (LQT2) because of impaired function of the pore-forming  $\alpha$ -subunit of the voltage-gated  $K_v11.1$  channel, which is a key player of repolarization in cardiac cells.<sup>3</sup> LQT2

is the second most common type of congenital LQTS, and is specifically characterized by notched T waves in the ECG<sup>4</sup> and a tendency to develop arrhythmias during sudden startling.<sup>5</sup>

Voltage-gated potassium ( $K_v$ ) channels also play a role in glucagon and insulin secretion from the pancreatic  $\alpha$  and  $\beta$  cells<sup>1,6–9</sup> and possibly also in the secretion of the incretin gut hormones, glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide (GIP), secreted from intestinal L and K cells, respectively.<sup>10</sup> These hormones are crucial for glucose regulation, and LQT1 patients have hyperinsulinemia and postprandial hypoglycemia.<sup>7</sup> Blockade of  $K_v11.1$  channels results in depolarization of the resting membrane potential and increases action potential firing rate by 32% (investigated by patch-clamp technique) and the release of insulin by 77% in human pancreatic  $\beta$  cells.<sup>9</sup> Blocking  $K_v11.1$  channels in  $\alpha$  cells impairs glucagon secretion.<sup>8</sup> Both conditions decrease glucose levels. Hypoglycemia is associated with increased propensity for QT prolongation and other adverse cardiovascular effects.<sup>11–13</sup>

Therefore, we investigated whether LQT2 patients with functional mutations in *hERG* and impaired  $K_v11.1$  channel function have increased glucose-stimulated insulin and incretin secretion and decreased levels of glucagon resulting in decreased glucose levels after oral glucose ingestion.

## METHODS

### Study Participants

Eleven LQT2 patients with loss-of-function mutations in *hERG* were recruited from the outpatient clinic at the Cardiology Department at Gentofte Hospital, Denmark. Two control subjects, matched to each individual patient with respect to body mass index (BMI), age, and sex, were recruited for examination in the present study from regional population-based studies, the Inter99, Health2006, 2010, or DanFund studies.<sup>14,15</sup> A computer algorithm, developed by a data manager independent of the research study, was applied to randomly select the control subjects based on their match with respect to sex,  $\pm 1$  BMI, and age ( $\pm 3$  years), inviting the closest matches first for participation in the study. Updated BMI and age were used for matching. Control participants were excluded if they were diagnosed with any known chronic disease, including diabetes mellitus, but were not screened for prediabetes because this could induce selection bias toward a falsely healthier metabolic phenotype given their BMI.

Before examination, all participants were fasting overnight and were free of any medication in the morning before examination. Ten of 11 LQT2 patients were on  $\beta$ -blocking agents, 7 had an implantable cardioverter-defibrillator, and 1 had a pacemaker.

### Ethics

Before participation, informed written consent was obtained from all participants. The project was approved by The

Committees on Health Research Ethics in the Capital Region of Denmark (reference number: H-4-2010-036) (institutional review board) and was performed in accordance to the Helsinki Declaration II. The participants gave informed consent, participation in the investigation was voluntary, and the individuals could retract their consent to participate at any time (ClinicalTrials.gov Identifier: NCT02775513).

Study approval for the animal study was obtained from the Danish Animal Experiments Inspectorate (2013-15-2934-00833) and the procedures followed were in accordance with institutional guidelines.

## Genetics

All patients were originally screened for functional mutations known to cause LQTS.<sup>16</sup> The LQT2 patients were all heterozygous carriers from 5 different families with the following functional missense mutations: *hERG* K101E (4 patients, grandmother, mother, son, and daughter), *hERG* I96T (1 patient), *hERG* F29L (2 patients, mother and son), *hERG* I400N (2 patients, mother and daughter), or *hERG* G572R (2 patients, aunt and niece).<sup>17,18</sup> The first 3 mutations mentioned are located in the Per-Arnt-Sim (PAS) domain, which contains a signal-sensing region and causes trafficking defects.<sup>19</sup> I400N is in the S1 transmembrane segment and disrupts the voltage-sensing unit.<sup>17</sup> G572R is in the S5 transmembrane segment and the pore-forming unit, and causes reduced activation of the channel or disturbs the channel's gating properties.<sup>18</sup>

## Oral Glucose Tolerance Test

Blood samples for measurements of plasma glucose, serum insulin, serum C-peptide, plasma total GLP-1, plasma total GIP, plasma glucagon, and serum potassium were taken after an overnight fast and during a 6-hour 75-g oral glucose tolerance test (OGTT). Fasting blood samples were taken 15, 10, and 0 minutes before glucose ingestion. Blood sampling was repeated every 15 minutes for the first hour and every half hour for the following 5 hours.

## Blood Samples

Plasma glucose was measured by a colorimetric assay on an automated Vitros 5.1 FS/5600 analyzer (Ortho Clinical Diagnostics) with a lower limit of quantitation of 19.8 mg/dL and intra- and interassay coefficients of variation of 0.025.

Hypoglycemic glucose values were defined as blood glucose <70 mg/dL. The generic nondiabetic glycemic threshold for impairment of cognitive function is <50 mg/dL.<sup>20</sup> This level was defined as serious hypoglycemia based on The International Hypoglycemia Group guidelines.<sup>20</sup>

Serum potassium was measured using Vitros 5.1 FS/5600 analyzer (Ortho Clinical Diagnostics). Intra- and interassay coefficients of variation were 0.05. The analytic detection limit was 1 mmol/L.

Serum insulin and C-peptide were both measured on an automated Cobas e411 analyzer (Roche). The analytic detection limit was 1.4 and 3 pmol/L with total intra- and interassay coefficients of variation of <0.04 and <0.025, respectively.

Plasma levels of total GLP-1, total GIP, and glucagon were measured with validated radioimmunoassays.<sup>21–23</sup> The assays have a detection limit of <2 pmol/L (1 pmol/L for glucagon)

and intra-assay and interassay coefficients of variation of <0.06 and 0.15, respectively.

## Electrocardiography

ECG recordings (MAC1600 ECG machine [GE Healthcare]) were made before each blood sampling: 5 minutes before glucose ingestion and repeated every 15 minutes for the first hour after glucose ingestion and then every half-hour for the following 5 hours. The mean of 5 consecutive recordings at each time point was used. QT intervals were corrected by heart rate with the Bazett formula ( $QTcB = QT/(RR)^{1/2}$ ), and the Fridericia formula ( $QTcF = QT/(RR)^{1/3}$ ).

## Hypoglycemia Questionnaire

An electronic questionnaire in the software SurveyXact was completed by all participants before examination. The questionnaire included an adapted standard questionnaire for symptoms of hypoglycemia.<sup>7</sup>

## Continuous Glucose Monitoring

Continuous blood glucose monitoring (CGM) was performed after the baseline examination using iPro2 (Medtronic) for a duration of between 3 and 7 days according to clinical recommendations<sup>24</sup> and following the manufacturer's manual. Only 6 of 11 LQT1 patients agreed to be investigated by CGM and were matched with 6 control participants. Results are shown in the [online-only Data Supplement Appendix](#) (online only Data Supplement Table I).

## Biochemical and Anthropometric Measures

Height and weight of LQT2 patients and control participants (without shoes and wearing light indoor clothes) were measured before examination; BMI was calculated as weight in kilograms divided by the square of height in meters ( $\text{kg}/\text{m}^2$ ). The percentage of fat was measured with a bioimpedance analyzer, Biodynamics BIA 310e (Biodynamics).

## OGTT in Rats

Twenty-two female Wistar rats were assigned to 2 weight-matched groups and received an intravenous injection under isoflurane anesthesia of dofetilide (5 mg/kg) or vehicle 15 minutes before glucose gavage feeding (2 g/kg) (time, 0 minutes). Further details are described in the [online-only Data Supplement Appendix](#).

## Cell Studies

siRNAs against *hERG* were obtained and acute stimulation with and without glucose (180 mg/dL) of cultured L (GLUTag) and  $\beta$  (Min-6) cells (with/without siRNA *hERG*), using ~80% confluent cells from different batch numbers ( $n=3$ ), were performed. Further details are described in the [online-only Data Supplement Appendix](#).

## Calculations

Total AUC was calculated as the total area under the curve with  $y=0$  as baseline. Incremental and decremental AUC, defined as



the AUC above or below fasting level (mean of time point −15, −10, and 0), respectively, were calculated in GraphPad Prism 5 (GraphPad Software Inc) using the trapezoidal method with the fasting levels as baseline for the time curve.

Responses were divided into 3 time intervals: the acute response (0–30 minutes), the standard 2-hour response (0–120 minutes), and the extended full 6-hour response (0–360 minutes) to 75-g glucose ingestion.

Insulinogenic index [(serum insulin at 30 minutes – fasting serum insulin) / (plasma glucose at 30 minutes – fasting plasma glucose)] was calculated as a measure of  $\beta$ -cell function and describes early phase insulin secretion. Whole-body insulin sensitivity was estimated from oral glucose tolerance data by applying the Matsuda insulin sensitivity index:  $(10\,000/\sqrt{(\text{fasting plasma glucose} \times \text{fasting serum insulin} \times \text{mean plasma glucose for the first 2 hours} \times \text{mean serum insulin for the first 2 hours})})$ . Insulin resistance was estimated with the fasting homeostasis model assessment of insulin resistance index (HOMA-IR) and calculated as follows:  $(\text{fasting plasma glucose} \times \text{fasting serum insulin})/405$ . Disposition index was calculated as insulinogenic index divided by HOMA-IR.  $\beta$ -Cell function corrected for insulin sensitivity was assessed by the Insulin Secretion Sensitivity Index-2 (ISSI-2). ISSI-2 is a validated OGTT-derived measure of  $\beta$ -cell function similar to the disposition index. ISSI-2 has been validated against the disposition index, calculated on the basis of a frequently sampled intravenous glucose tolerance test, with which it exhibits a stronger correlation than other OGTT-derived measures of  $\beta$ -cell function. ISSI-2 is defined as the product of insulin secretion as measured by the ratio of  $\frac{\text{incremental AUC}_{\text{insulin0-120}}}{\text{incremental AUC}_{\text{glucose0-120}}}$  to insulin sensitivity as measured by the Matsuda index:  $\text{Matsuda Index} \times \left( \frac{\text{incremental AUC}_{\text{Ins0-120}}}{\text{incremental AUC}_{\text{Gluc0-120}}} \right)^{.25,26}$ .

The frequency and severity of hypoglycemia symptoms were quantified by analyzing the adapted hypoglycemia questionnaire.<sup>7</sup> The total frequency and severity score was calculated for each participant as the sum of points for all frequency and severity questions.

The acute cardiac responses to glucose ingestion evaluated as QTcB and QTcF in the recorded ECGs were found by analyzing the maximum  $\Delta$  increase within the first 30 minutes of the OGTT and subtracting the fasting value to analyze response differences between patients and healthy controls.

## Sample Size Calculation

Our prior data on LQT1 patients<sup>7</sup> indicated that a difference of 2000 pmol/L $\times$ min in the  $\frac{\text{incremental AUC}_{(0-30)}}{\text{incremental AUC}_{(0-30)}}$  insulin response between 11 matched pairs would reject the null hypothesis that this response difference is zero with power 0.8,  $P<0.05$ . With our present data on 11 patients and 22 matched controls and a difference of 2471 pmol/L $\times$ min $\pm$ 489, we have a power  $>0.9$ ,  $P<0.05$  (paired design).

## Statistics

Prespecified primary variables were area under the curve (AUC) for hormones and glucose.

Statistical analyses were made in SAS Enterprise Guide 7.11, SAS institute. Data distribution was studied before further analyses and insulin data were consequently log-transformed.

Mixed-model analysis of variance (ANOVA) with family mutation as an additional factor was performed on patient group data. There were no significant differences between the 5 families. Consequently, data were analyzed by using mixed-model ANOVA contrasting LQT2 patients versus control participants in matching pairs.

The Fisher exact test was used to analyze the difference in occurrence of hypoglycemic plasma glucose values between patients and matched control participants during the OGTT.

Differences in fasting,  $\frac{\text{decremental AUC}_{0-120\text{min}}}{\text{decremental AUC}_{0-120\text{min}}}$  and  $\frac{\text{incremental AUC}_{120-360\text{min}}}{\text{incremental AUC}_{120-360\text{min}}}$  glucagon responses of hypoglycemic versus nonhypoglycemic patients, were analyzed with unpaired  $t$  tests.

Questionnaire data were quantified as numeric categorical data, and differences in mean scores between patients and control participants were tested with a paired  $t$  test.

Changes in QTcB, QTcF, and heart rate from baseline during the OGTT were analyzed with a proc mixed ANOVA to study cardiac repolarization disturbances after glucose stimulation, and, if interaction between time and group was identified, the groups were subsequently analyzed separately.

Data are presented as mean $\pm$ standard error of the mean or geometric mean (95% confidence interval) for log-transformed data. A  $P$  value of  $<0.05$  was considered statistically significant.

## RESULTS

### Baseline Characteristics

The patients with LQT2 and the healthy control participants were successfully matched with regard to sex, age, BMI, and fat percentage and heart rate, as well (Table 1). LQT2 patients had longer QTcB and QTcF intervals than control participants (Table 1).

### Fasting Circulating Hormone Levels

Patients with LQT2 had 32% lower fasting plasma levels of glucagon in comparison with control participants

**Table 1. Subject Characteristics**

Characteristics	Long-QT Syndrome Type 2 Patients	Control Participants	$P_{\text{patient vs control}}$
Total no. (men/women)	11 (2/9)	22 (4/18)	
Age, y	42 $\pm$ 6	42 $\pm$ 4	1.0
Body mass index, kg/m <sup>2</sup>	23.4 $\pm$ 0.8	24.0 $\pm$ 0.7	0.6
Fat, %	26.5 $\pm$ 1.8	26.8 $\pm$ 1.9	0.9
Fasting QTcB, ms	477 $\pm$ 7	419 $\pm$ 6	$<0.0001$
Fasting QTcF, ms	485 $\pm$ 8	423 $\pm$ 7	$<0.0001$
Fasting heart rate, beats/min	55.0 $\pm$ 2.8	57.8 $\pm$ 1.2	0.4

Subject characteristics were analyzed with a mixed-model analysis of variance. Data are shown as mean $\pm$ standard error of the mean.

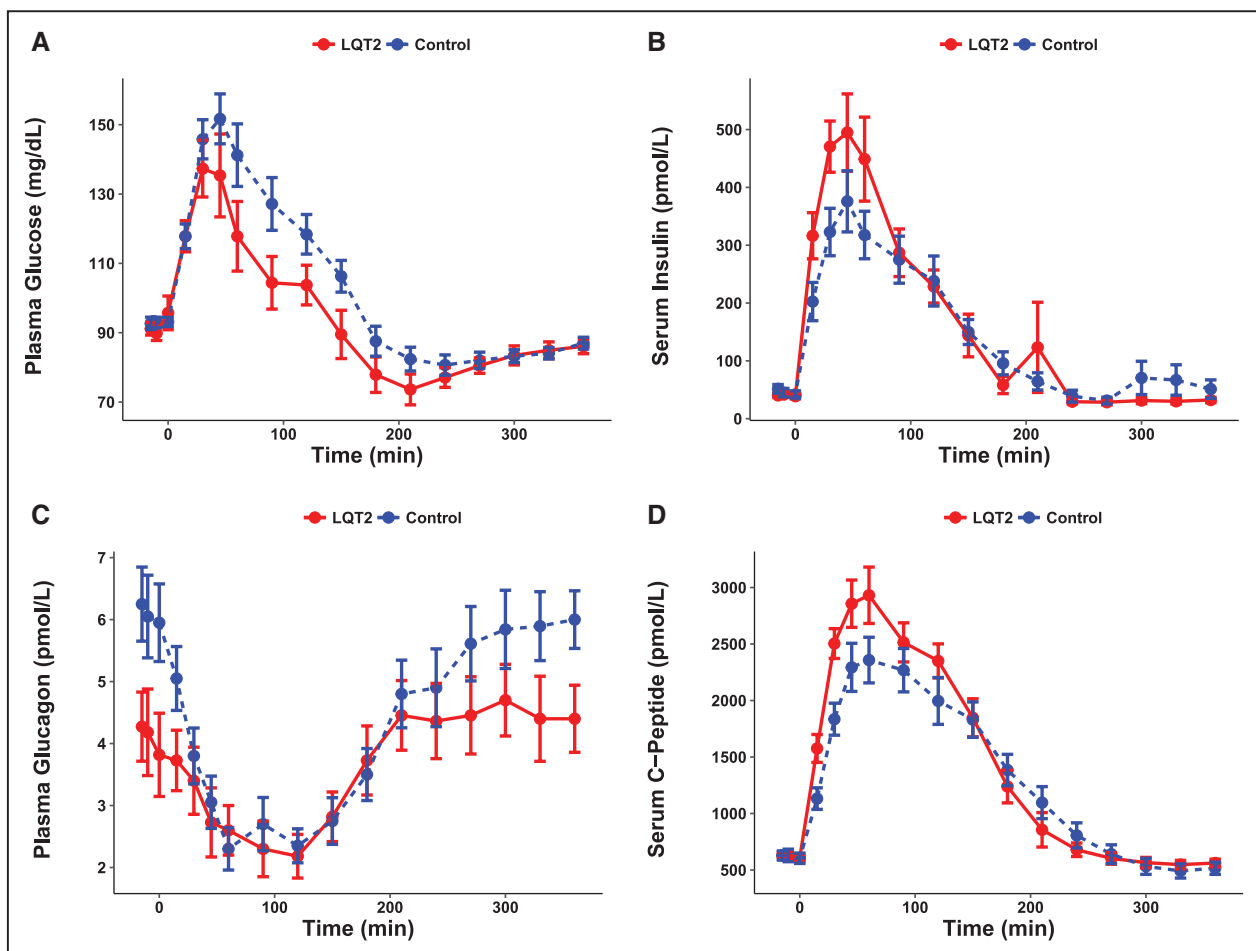
( $4.1 \pm 0.8$  versus  $6.0 \pm 0.7$  pmol/L,  $P=0.02$ ). There were no differences between LQT2 patients and control participants in fasting levels of plasma glucose, serum insulin, serum C-peptide, plasma GLP-1, plasma GIP, or serum potassium (Figures 1 and 2).

There were no differences in fasting hemoglobin A1c ( $33.8 \pm 1.2$  [range, 28–38] versus  $34.8 \pm 1.0$  [range, 29–44 mmol/mol])  $P=0.4$ , fasting hemoglobin ( $8.2 \pm 0.1$  versus  $8.4 \pm 0.1$  mmol/L)  $P=0.1$ , fasting total cholesterol ( $4.8 \pm 0.4$  versus  $4.7 \pm 0.3$  mmol/L)  $P=0.7$ , or fasting creatinine ( $64.6 \pm 3.1$  versus  $64.7 \pm 2.2$   $\mu$ mol/L)  $P=1.0$  between groups. None of the participants had hemoglobin A1c levels  $\geq 48$  mmol/mol (definition of diabetes mellitus).

### Glucose-Stimulated Hormone Responses

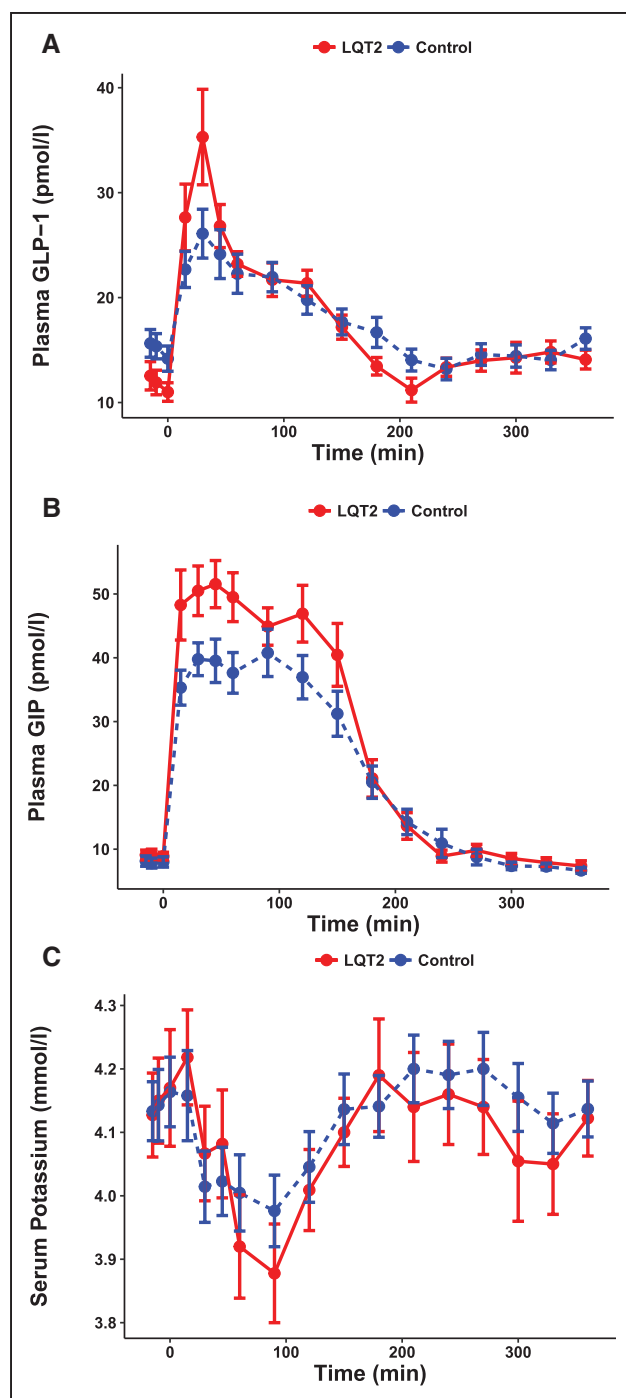
LQT2 patients had lower glucose levels during the 6-hour OGTT than control participants (mean glucose during the 6 hours was  $97 \pm 4$  mg/dL versus  $106 \pm 2$  mg/dL,  $P=0.02$  with a 16% smaller 2-hour total AUC,  $P=0.03$ ,

and 6-hour total AUC,  $P=0.02$  (Table 2, Figure 1A). A higher proportion of patients with LQT2 developed hypoglycemia (plasma glucose levels  $<70$  mg/dL) during the OGTT than control participants (7/11=63% versus 8/22=36%, respectively), although the difference was not significant given the sample size ( $P=0.16$  by Fisher exact test). During the 6-hour OGTT with 17 measurements, the 11 patients had 16 hypoglycemic measurements in comparison with 13 hypoglycemic measurements in the 22 matched control participants. Two patients with LQT2, but no healthy participants, developed serious hypoglycemia (plasma glucose  $<50$  mg/dL) at 3 time points during the OGTT (lowest registered plasma glucose was 43 mg/dL after 150 minutes and 45 mg/dL after 180 minutes, respectively),  $P=0.04$ . Six of 7 hypoglycemic LQT2 patients had mutations (F29L, K101E, and 196T) in the PAS domain (including the 2 with serious hypoglycemia [196T and K101E]) in contrast to the 4 nonhypoglycemic LQT2 patients where 3 of 4 had mutations in the transmembrane segment S1 or S5.



**Figure 1. Plasma glucose and hormone responses to oral glucose ingestion in 11 LQT2 patients and 22 matched healthy control participants.**

Glucose (A), insulin (B), glucagon (C), and C-peptide (D) fluctuations during a 6-hour oral 75-g glucose tolerance test, shown as mean  $\pm$  SEM for non-log-transformed data. LQT2 patients, red full lines; control participants, blue broken lines. LQT2 indicates long-QT syndrome type 2; and SEM, standard error of the mean.



**Figure 2. Incretin and potassium responses to oral glucose ingestion in 11 LQT2 patients and 22 matched healthy control participants.**

GLP-1 (A), GIP (B), and potassium (C) fluctuations during a 6-hour oral 75-g glucose tolerance test, presented as mean $\pm$ SEM. LQT2 patients, red full lines; control participants, blue broken lines. GIP indicates glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; LQT2, long-QT syndrome type 2; and SEM, standard error of the mean.

LQT2 patients had increased serum insulin and C-peptide responses to oral glucose. Thus, 30-minute insulin and C-peptide responses (incremental AUC<sub>(0–30)</sub>) were 57% and

60% higher in patients with LQT2 than in matched control participants ( $P=0.03$  and  $0.004$ ), along with increased C-peptide after 2 hours (incremental AUC<sub>(0–120)</sub>,  $P=0.03$ ) (Table 2, Figure 1B and 1D).

LQT2 patients had also increased incretin responses to oral glucose. Thus, GLP-1 responses were 56% to 94% higher in patients than in controls at 30 minutes, 2 hours, and 6 hours (incremental AUC,  $P=0.01$ – $0.003$ ). The 2-hour response of GIP was increased by 78% in comparison with controls ( $P=0.02$ ) (Table 2, Figure 2A and 2B).

There was no difference in potassium responses to glucose between the groups,  $P>0.1$  (Figure 2C).

During the OGTT, the LQT2 patients had lower glucagon response to low glucose on oral glucose stimulation (decremental AUC<sub>(0–360)</sub>,  $P=0.008$ , Table 2) than controls (Figure 1C). Furthermore, the 7 of 11 LQT2 patients that developed hypoglycemic plasma glucose levels  $<70$  mg/dL during the OGTT had lower glucagon response to low glucose levels during the OGTT than the 4 patients that did not develop hypoglycemia (incremental AUC<sub>(120–360)</sub>  $285\pm59$  versus  $648\pm28$ ,  $P=0.004$ ). Fasting and decremental AUC<sub>(0–120)</sub> glucagon was  $3.6\pm2.6$  versus  $4.9\pm1.7$ ,  $P=0.07$ , and  $134\pm89$  versus  $269\pm54$ ,  $P=0.1$ , respectively Figure 3.

$\beta$ -Cell function measured by insulinogenic index was 167% increased in LQT2 patients in comparison with control participants ( $P=0.001$ , Figure 4A). There was no difference between patients and control participants regarding insulin sensitivity measured by the Matsuda index (geometric mean values [95% confidence interval],  $16.0$  [ $12.6$ – $20.3$ ] versus  $18.3$  [ $14.1$ – $23.7$ ],  $P=0.5$ ) or insulin resistance measured by HOMA-IR ( $1.3$  [ $1.0$ – $1.6$ ] versus  $1.2$  [ $0.9$ – $1.7$ ] (mg/dL) $\times$ (mIU/mL),  $P=0.9$ ). The insulin sensitivity-adjusted  $\beta$ -cell function in LQT2 patients, measured by ISSI-2, was 2-fold higher than in control participants with geometric mean values [95% confidence interval] for patients with LQT2 of  $4398$  [ $2259$ – $8562$ ] versus control participants  $2156$  [ $1961$ – $3201$ ],  $P=0.03$  (Figure 4B).  $\beta$ -Cell function measured by the disposition index was 233% higher in *hERG* patients ( $23.5$  [ $11.8$ – $46.8$ ] versus  $10.1$  [ $5.3$ – $17.0$ ] in control participants,  $P=0.002$ ).

### Glucose-Stimulated ECG Responses

The duration of QTcB and QTcF was longer in LQT2 patients than in control participants during the 6-hour OGTT (Figure 5),  $P<0.0001$ .

Glucose ingestion led to a biphasic QTc and heart rate response. QTc increased in both groups after 15 to 45 minutes, and was increased again 300 minutes after glucose ingestion,  $P<0.05$  to  $0.0001$  (Figure 5). The maximum acute increase of QTc (0–30 minutes) was  $77_{(\text{for QTcB})}$  to  $122_{(\text{for QTcF})}$  % larger in patients with LQT2, in comparison with control participants,  $P=0.01$  to  $0.004$ , whereas the

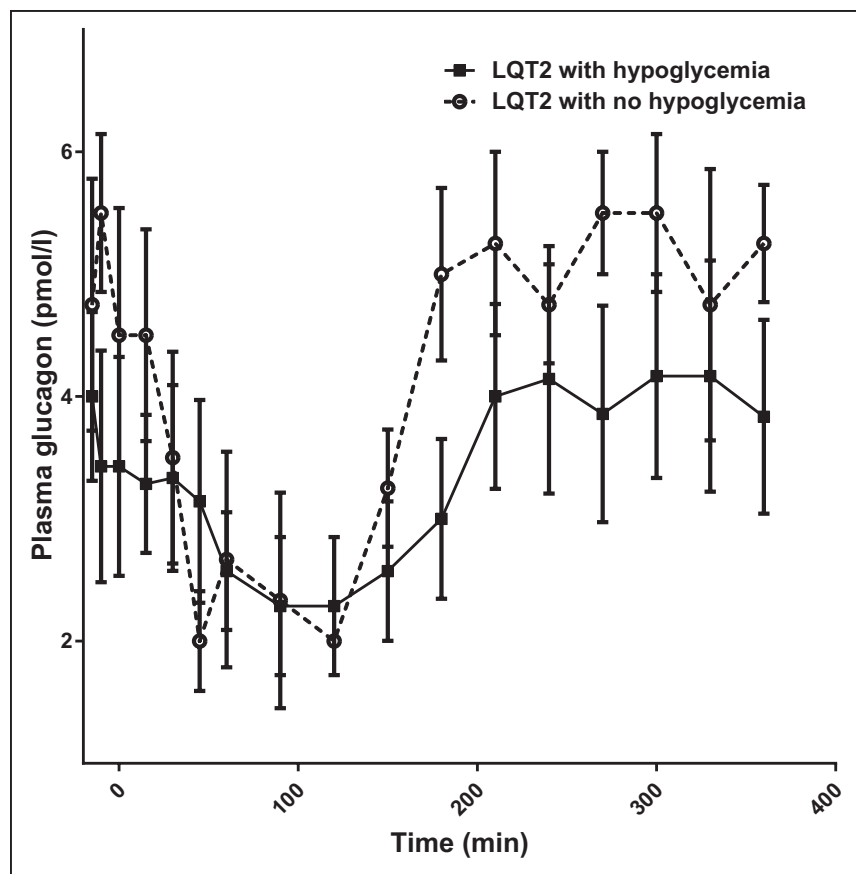
**Table 2. Hormone and Glucose Responses During Oral Glucose Tolerance Test**

	Long-QT Syndrome Type 2 Patients	Control Participants	P Value
Total area under the curve			
0–120 min			
C-peptide (pmol/L×min)	275 973±22 554	233 846±17 455	0.1
GIP (pmol/L×min)	5195±463	4376±376	0.1
GLP-1 (pmol/L×min)	2774±250	2617±187	0.6
Glucagon (pmol/L×min)	323±53	388±41	0.3
Glucose (mg/dL×min)	13 242±865	15 710±613	0.03*
Insulin (pmol/L×min)	40 107±4947	32 694±3498	0.2
0–360 min			
C-peptide (pmol/L×min)	509 764±47 780	472 385±40 052	0.4
GIP (pmol/L×min)	9320±772	8004±607	0.1
GLP-1 (pmol/L×min in)	6354±458	6206±326	0.9
Glucagon (pmol/L×min)	1258±177	1460±142	0.3
Glucose (mg/dL×min)	33 113±1261	36 590±937	0.02*
Insulin (pmol/L×min)	56 954±7863	52 260±5600	0.6
Incremental and decremental area under the curve			
0–30 min			
C-peptide (pmol/L×min)	26 613±2546	16 645±1800	0.004*
GIP (pmol/L×min)	845±86	650±61	0.07
GLP-1 (pmol/L×min)	383±57	197±40	0.01*
Glucagon (pmol/L×min)	−21.2±7.9	−38.2±7.2	0.02*
Glucose (mg/dL×min)	712±85	778±106	0.6
Insulin (pmol/L×min)	6786±843	4315±596	0.03*
0–120 min			
C-peptide (pmol/L×min)	210 371±17 888	160 148±13 085	0.03*
GIP (pmol/L×min)	4437±394	3451±328	0.02*
GLP-1 (pmol/L×min)	1521±199	851±161	0.003*
Glucagon (pmol/L×min)	−183±62	−340±55	0.01*
Glucose (mg/dL×min)	3027±829	4630±595	0.1
Insulin (pmol/L×min)	35 707±4263	27 182±3015	0.1
0–360 min			
C-peptide (pmol/L×min)	304 073±30 777	264 009±25 202	0.2
GIP (pmol/L×min)	6567±729	5418±576	0.2
GLP-1 (pmol/L×min)	2284±324	1334±258	0.01*
Glucagon (pmol/L×min)	−334±160	−761±142	0.008*
Glucose (mg/dL×min)	3549±1009	5729±721	0.09
Insulin (pmol/L×min)	44 250±6175	38 658±4466	0.5

Two-hour and 6-h total area under the curve and incremental and decremental acute (0–30 min), 2 h (0–120 min), and full (0–360 min) area under the curve hormone response during OGTT in 11 LQT2 patients and 22 matched control participants analyzed with a mixed-model analysis of variance. Data are shown as mean±standard error of the mean. GIP, glucose-dependent insulintropic polypeptide; GLP-1, glucagon-like peptide-1; LQT2, long-QT syndrome type 2; and OGTT, oral glucose tolerance test.

\* $P<0.05$ .





**Figure 3. Plasma glucagon response to oral glucose among LQT2 patients with (n=7) and without (n=4) hypoglycemia.**

The 7 of 11 LQT2 patients that developed hypoglycemic plasma glucose levels <70 mg/dL during the OGTT had lower glucagon response to low glucose levels during the OGTT than the 4 patients that did not develop hypoglycemia (incremental AUC<sub>120-360</sub> 285±59 versus 648±28,  $P=0.004$ ) analyzed with an independent *t* test. LQT2 patients with hypoglycemia, full lines; LQT2 patients without hypoglycemia, broken lines. Data are shown as mean±SEM. AUC indicates area under the curve; LQT2, long-QT syndrome type 2; OGTT, oral glucose tolerance test; and SEM, standard error of the mean.

heart rate increase was similar between groups (Figure 5 and online-only Data Supplement Table II).

### Questionnaire

LQT2 patients reported more symptoms of hypoglycemia ( $23.8\pm2.2$  versus  $19.3\pm1.5$  points,  $P=0.04$ ; Figure 6) and experienced more severe symptoms, if a meal was missed, and more often reported feeling uncomfortable a couple of hours after a meal (online-only Data Supplement Table III). Furthermore, patients with LQT2 experienced more frequent and severe symptoms if they suddenly stood up from a sitting position, and reported increased frequency of heart palpitations, and occasional fainting, as well.

### Pharmacological Blockage of Kv11.1 During OGTT in Rats

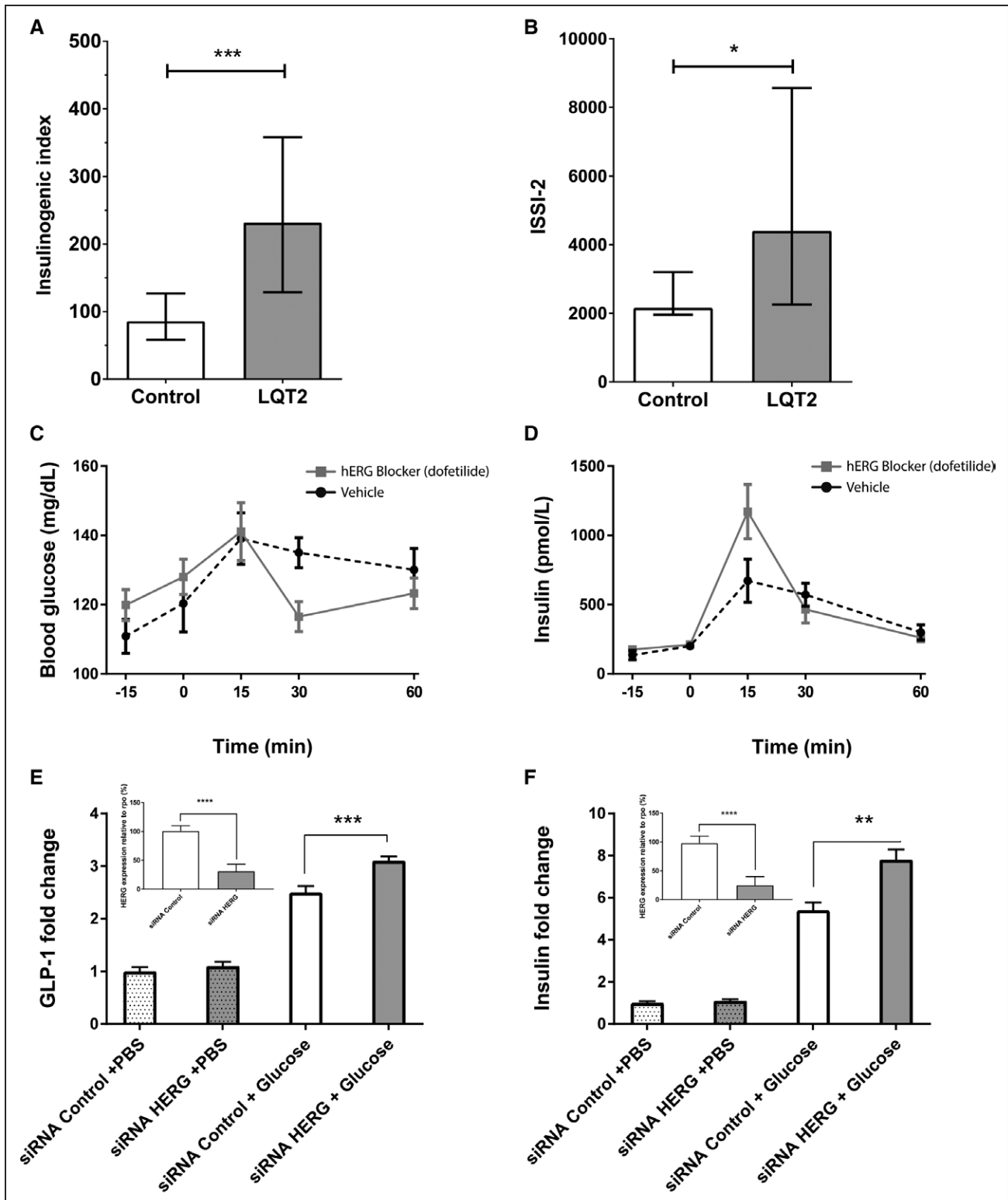
Rats receiving the selective Kv11.1 channel blocker dofetilide (antiarrhythmic drug)<sup>27,28</sup> had increased insulin secretion, and corresponding reduced blood glucose levels after glucose intake in comparison with rats receiving vehicle,  $P<0.01$  (Figure 4C and 4D). Sixty-three percent of rats receiving dofetilide developed glucose values lower than their fasting levels during the 1-hour OGTT in comparison with 25% of rats in the vehicle group.

### hERG Blockage in Cultured L and $\beta$ -Cells

Glucose-induced GLP-1 secretion was significantly higher (24%,  $P=0.009$ ) in cultured L cells with knock-down of hERG expression (siRNA<sub>hERG</sub>) in comparison with control cells (siRNA<sub>mock</sub>). Glucose-induced insulin secretion was significantly higher (54%,  $P=0.001$ ) in cultured  $\beta$  cells with knockdown of hERG expression (siRNA<sub>hERG</sub>) in comparison with control cells (siRNA<sub>mock</sub>) (Figure 4E and 4F).

### DISCUSSION

Here, we show that besides a prolonged cardiac repolarization phase, patients with LQT2 caused by hERG mutations display exaggerated incretin and endocrine pancreatic function with >50% increases in GLP-1, GIP, and insulin secretion, and defective glucagon secretion, causing low plasma glucose levels and increased risk of symptomatic hypoglycemia following glucose ingestion. Pharmacological Kv11.1 blockade (dofetilide) in rats had similar effects and inhibition of hERG in  $\beta$  and L cells directly increased insulin and GLP-1 secretion up to 50%. Furthermore, glucose ingestion aggravated cardiac repolarization disturbances in LQT2 patients and prolonged the cardiac repolarization phase in healthy controls.



**Figure 4. Inhibition of *hERG* in humans, rats, and cells.**

Differences in  $\beta$ -cell function in response to oral glucose ingestion in 11 LQT2 patients and 22 matched healthy control participants.  $\beta$ -Cell function measured by insulinogenic index (**A**) and Insulin Secretion-Sensitivity Index-2 (ISSI-2), which is a validated OGTT-derived measure of insulin sensitivity-adjusted  $\beta$ -cell function (**B**) analyzed with a mixed-model ANOVA. Insulinogenic index and ISSI-2 are shown as geometric means [95% CI]. **C** and **D**, Oral glucose tolerance test in rats. Rats receiving the selective Kv11.1 channel blocker dofetilide (antiarrhythmic drug) ( $n=8$ ) had increased insulin secretion and reduced blood glucose (incremental  $AUC_{0-60}$  439 $\pm$ 182 versus 1218 $\pm$ 175) after glucose intake in comparison with rats receiving vehicle ( $n=14$ ),  $P<0.01$ . (Continued)

## Functional Role of $K_v11.1$ Channels in Human Glucose Regulation

Until now, the functional role of  $K_v11.1$  channels in human glucose regulation has been unknown, although several endocrine cell studies have shown that  $K_v11.1$  is expressed in  $\alpha$  (glucagon),  $\beta$  (insulin),<sup>1,6,8,9</sup> L (GLP-1),<sup>10</sup> and likely K cells (GIP) (identified by microarray, personal communication, Fiona Gribble, Cambridge University, UK, 2016) in the pancreas and intestines, respectively. Our results show that reduced function of  $K_v11.1$  is associated with acute hyperinsulinemia, >50% increased GLP-1 and GIP levels, and correspondingly lower plasma glucose levels after a standard oral 75-g glucose load. The LQT2 patients had 2-fold higher  $\beta$ -cell function, ie, the ability to secrete more insulin on glucose ingestion, also when taking the insulin sensitivity status into account. We also show that inhibition of *hERG* directly increases secretion of both insulin and GLP-1 from cultured  $\beta$  and L cells, respectively. These results combined with previously published electrophysiology studies of *hERG* blockade in human  $\beta$  cells<sup>8,9</sup> support that blocking  $K_v11.1$  has a direct effect on both insulin- and GLP-1-producing cells. Thus, although a nonincretin-dependent test of  $\beta$ -cell function (eg, an intravenous glucose tolerance test) was not performed, the hyperinsulinemia in *hERG* mutation carriers seems to be attributable to a dual effect: a direct stimulatory effect on  $\beta$  cells causing increased insulin secretion, and an indirect effect attributable to increased secretion of GLP-1 (and GIP) from L (– and K) cells, which in turn also stimulates insulin secretion and thereby lowers blood glucose.

The glucagon levels were lower in LQT2 patients than in matched control participants, even though they had lower glucose levels, which is an indication of distorted  $\alpha$ -cell function, because, under normal physiological circumstances, hypoglycemia would be expected to increase glucagon levels to counteract the hypoglycemia. Indeed, the hypoglycemic LQT2 patients had an inappropriately low increase in glucagon during low glucose levels in comparison with the nonhypoglycemic LQT2 patients. Furthermore, inhibition of  $K_v11.1$  channels in  $\alpha$  cells leads to an inhibition of glucagon secretion.<sup>8</sup> Exocytosis in  $\alpha$  cells is caused by membrane potential-dependent opening of the  $Ca_v$  channels mediating the calcium entry. With inhibition of the  $K_v$  current, there is no reactivation of the  $Na_v$  channels and  $Ca_v$  channels leading to a marked reduction of the action potential amplitude, thereby inhibiting glucagon release. As a consequence, glucagon secretion

is decreased when  $K_v$  channels are blocked<sup>8,29,30</sup> or dysfunctional as in LQT2 patients. Therefore, LQT2 patients have impaired counterregulatory defense against hypoglycemia. Glucagon was measured with a method recently validated versus mass spectrometry and has a detection limit of 1 pmol/L. We therefore consider the measured plasma levels of glucagon to be accurate.<sup>31</sup>

## Clinical Implications of Higher Risk of Hypoglycemia

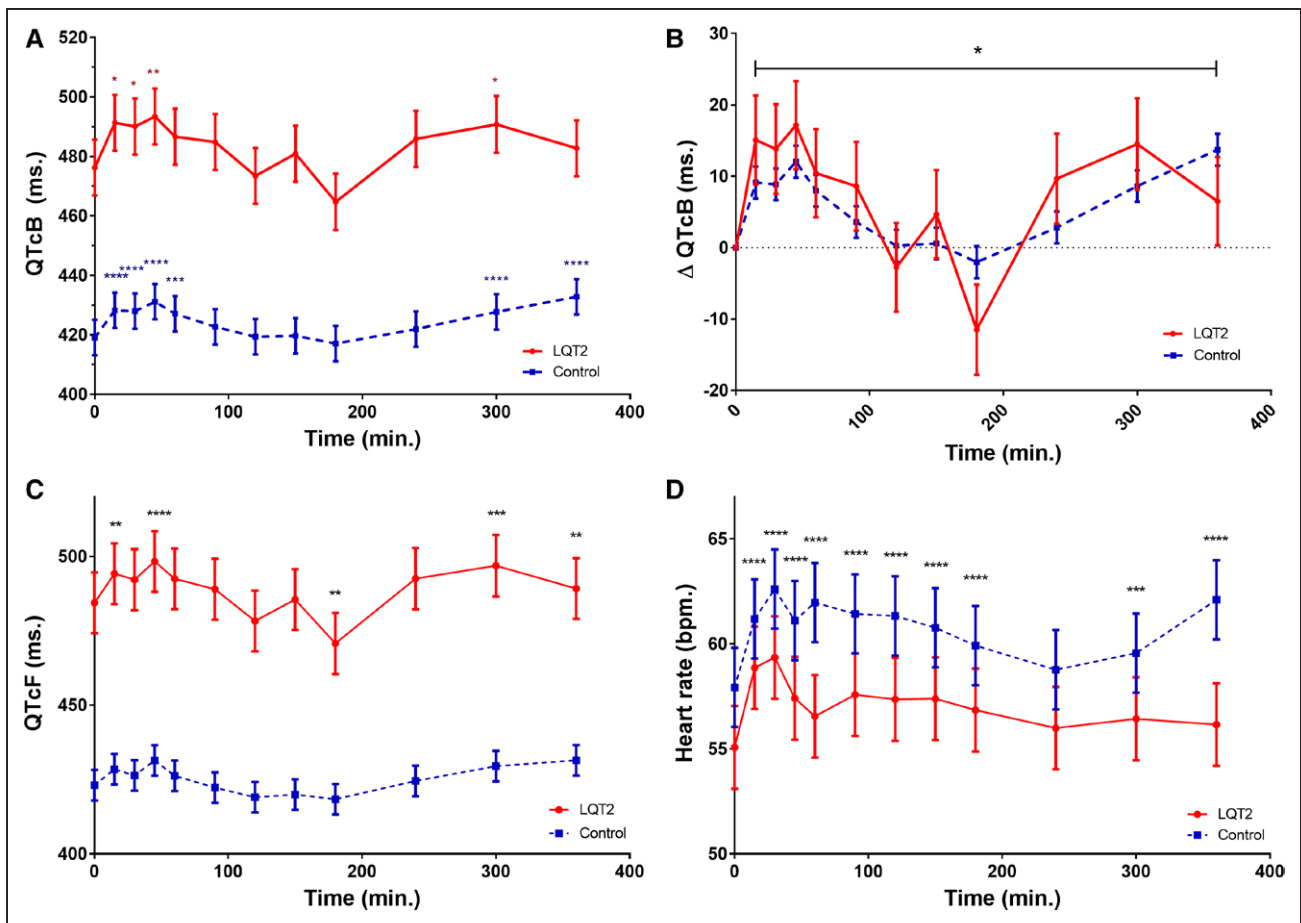
During the 6-hour OGTT, 63% of LQT2 patients became hypoglycemic, including 18% with serious hypoglycemia (<50 mg/dL glucose) versus 36% control participants among whom none became seriously hypoglycemic. Patients with LQT2 reported more symptoms of hypoglycemia in daily life, such as discomfort if a meal was missed, and discomfort some hours after meal intake, as well, in comparison with control participants. Some symptoms of hypoglycemia, like palpitations, are overlapping with symptoms of LQTS.

Hypoglycemia is associated with increased propensity for QT prolongation and other adverse cardiovascular effects.<sup>11–13</sup> Furthermore, a high-baseline QTc interval seems to be an important predictor for hypoglycemia-induced QTc prolongation.<sup>32</sup> The combination of symptomatic reactive hypoglycemia with LQT2 may therefore further increase the propensity for cardiac events in patients with LQT2. In line with these observations, children with LQT2 were found to have higher risk of developing severe hypoglycemia in a large retrospective study that called for awareness of this.<sup>33</sup> Because the study was retrospective, analyzing historical hospital data,<sup>33</sup> oral glucose tolerance tests were not performed. Thus, it is unknown whether the children also experienced reactive hypoglycemia.

Our findings further suggest that, similar to functional *KCNQ1* mutations,<sup>7</sup> functional *hERG* mutations may underlie some cases of essential postprandial hypoglycemia. This syndrome is characterized by appearance of reactive hypoglycemia after food intake without conspicuous cause. Thus, ECG monitoring and genetic testing could be considered when other causes of reactive hypoglycemia (eg, gastrointestinal surgery or medications) have been excluded.

An additional important finding in our study is that an increase of plasma glucose by only 54 to 72 mg/

**Figure 4 Continued.** Analyzed by an independent *t* test. **E** and **F**, Cultured  $\beta$ - and L-cell insulin and GLP-1 responses to glucose on *hERG* inhibition with siRNA. Glucose-induced GLP-1 secretion was significantly higher (24%,  $P=0.009$ ) in cultured L cells with knockdown of *hERG* expression (siRNA<sub>*hERG*</sub>) in comparison with control (siRNA<sub>mock</sub>) (siRNA knockdown efficiency=70±12%). Glucose-induced insulin secretion was significantly higher (54%,  $P=0.001$ ) in cultured  $\beta$  cells with knockdown of *hERG* expression (siRNA<sub>*hERG*</sub>) in comparison with control (siRNA<sub>mock</sub>) (siRNA knockdown efficiency=75±8%). Analyzed with 1-way ANOVA with Sidak correction. Data are shown as mean±SEM. \* $P<0.05$ . \*\* $P<0.01$ . \*\*\* $P<0.001$ . \*\*\*\* $P<0.0001$ . ANOVA indicates analysis of variance; AUC, area under the curve; CI, confidence interval; GLP-1, glucagon-like peptide-1; LQT2, long-QT syndrome type 2; OGTT, oral glucose tolerance test; PBS, phosphate-buffered saline; SEM, standard error of the mean; and siRNA, small interfering RNA.



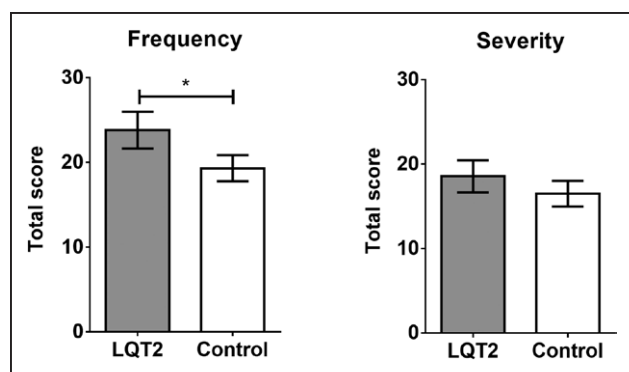
**Figure 5. QTc and heart rate changes during a 75-g oral glucose challenge in 11 LQT2 patients and 22 matched control participants.**

Changes in QTcB (asterisks indicate significant change from baseline and are shown for groups separated because of interaction between time and group) (A) and  $\Delta$  QTcB changes from baseline shown for both groups QTcB (asterisks indicate interaction between groups) (B). Changes in QTcF and heart rate (asterisks indicate significant change from baseline for groups analyzed together because there were no interactions between groups) (C and D) analyzed with a mixed-model ANOVA. LQT2 patients, red full lines; control participants, blue broken lines. Data are shown as means $\pm$ SEM. \* $P$ <0.05. \*\* $P$ <0.01. \*\*\* $P$ <0.001. \*\*\*\* $P$ <0.0001. LQT2 indicates long-QT syndrome type 2; and SEM, standard error of the mean.

dL during the OGTT affected heart rate and cardiac repolarization in both LQT2 patients and healthy control participants. However, the disturbances were more pronounced in patients with LQT2. Because LQT2 patients have a prolonged QT interval, they are more vulnerable to further increases in their QT interval than healthy individuals.<sup>34</sup> Interestingly, patients with both type 1 and type 2 diabetes mellitus experience prolonged QT interval because of a downregulation of  $K_{v11.1}$  channels<sup>35,36</sup> and because plasma glucose levels influence the normal  $K_{v11.1}$  channel function via ATP.<sup>9–11</sup> During hyperglycemia, increased cell metabolism will increase ATP production and thereby close the  $K_{ATP}$  channels. In cardiomyocytes, this may prolong the repolarization phase and thus increase QT interval, also in healthy individuals. However, patients with LQT2 seem to be even more vulnerable to cardiac events in response to glycemic excursions, which, by disturbing repolarization further, increase the

risk of QT dispersion and possible malignant arrhythmias. During the prolonged OGTT, a biphasic QTc curve was observed, high during peak glucose, lowest at 180 minutes, but then rising again. The lowest glucose levels were observed at 200 minutes, but thereafter remained low. Thus, the net result of glucose ingestion seems to be QT prolongation both during hyperglycemia, but also during the prolonged, relatively hypoglycemic state 5 hours after glucose ingestion.

There were no differences in fasting hemoglobin A1c or CGM between groups (CGM was only performed in half of the patients and not in any of the seriously hypoglycemic patients). This likely reflects that the fasting glucose levels were similar between the groups and that only a small fraction of the day is spent in a postprandial state where the differences between groups were pronounced. Furthermore, because patients with LQT2 reported more symptoms of hypoglycemia in daily life (discomfort if a



**Figure 6. Symptoms of hypoglycemia frequency and severity total score in 11 LQT2 patients and 22 matched control participants.**

Data were analyzed with a paired *t* test and shown as mean±SEM. \**P*=0.04. See analysis of all questions in [online-only Data Supplement Table III](#). LQT2 indicates long-QT syndrome type 2; and SEM, standard error of the mean.

meal was missed and discomfort some hours after meal intake), some LQT2 patients may unwittingly regulate their eating habits to avoid serious hypoglycemia. Also, a known bias when monitoring glucose levels with a CGM is that study participants may adjust behavior toward less glucose intake and thereby reduce the risk of reactive hypoglycemia during the CGM test days.

Nonetheless, the glucose ingested, equivalent of a medium-size sugar-containing soft drink, did lead to reactive hypoglycemia in the majority of LQT2 patients with 18% experiencing serious hypoglycemia (<50 mg/dL glucose). The hypoglycemia seemed to be coupled especially to mutations in the PAS domain. Severity analysis has identified the PAS domain of *hERG* as a region where mutations increase susceptibility to disease dramatically<sup>37</sup> by causing severe trafficking defects.<sup>19</sup> Serious incidences of hypoglycemia have previously been observed both in children with LQT2<sup>33</sup> and in LQT1.<sup>7</sup> Our demonstration that LQT2 patients, also in the absence of  $\beta$ -blockers, have increased risk of reactive hypoglycemia should lead to a greater awareness of the risk of glucose-induced hypoglycemia in LQTS. The risk of reactive hypoglycemia can be lessened by reducing the intake of easily digestible carbohydrates. Thus, overall, among patients with congenital LQTS, hypoglycemia may prove to be more clinically relevant than currently appreciated; however, the association with QT-related arrhythmias remains to be demonstrated.

### Medications That Affect Kv11.1 Channels and Risk of Hypoglycemia

We here show that the selective Kv11.1 channel blocker dofetilide,<sup>27,28</sup> which is used as an antiarrhythmic drug, increases insulin secretion and lowers blood glucose after glucose intake in rats. Similarly, the widely used fluoroqui-

nolone antibiotics that also block the Kv11.1<sup>1</sup> have been associated with hypoglycemia both in patients with and without diabetes mellitus, and with fatal hypoglycemia, as well, when used together with sulfonylurea.<sup>1,38,39</sup> Indeed, several widely used pharmaceutical agents are known to block the Kv11.1 channel,<sup>1,27</sup> and may, therefore, on the background of the present observations, also be suspected to increase the risk of reactive hypoglycemia.

### GLP-1 and Hypoglycemia

In glucose-tolerant individuals, elevated postprandial GLP-1 responses are associated with reactive hypoglycemia, which can be pronounced<sup>40,41</sup>; for instance, after gastric bypass surgery.<sup>42</sup> Furthermore, GLP-1-induced inhibition of glucagon secretion may aggravate hypoglycemia in such cases.<sup>40</sup> Thus, the increased secretion of GLP-1 in LQT2 patients is likely to contribute to the increased risk of hypoglycemia.

### $\beta$ -Blockers and Hypoglycemia

Ten of 11 LQT2 patients were on  $\beta$ -blocking agents, but were free of medication in the morning before examination. Thus, with 24 hours since the last dose and with a half-life of 3 hours, the concentration during the OGTT was minimal. In addition, previous human studies have shown that  $\beta$ -blocking agents have either no effect or a slightly increasing effect on blood glucose,<sup>7,43,44</sup> contrasting to the decreased glucose levels observed in the patients with LQT2, thus ruling out that the lower glucose levels among LQT2 in comparison with control participants could be attributable to  $\beta$ -blocking agents. Actually, prior use may even have positive effects on recovery from hypoglycemia,<sup>12</sup> and a recent study of diabetic patients showed that prior use of  $\beta$ -blockers led to lower incidence of severe hypertension and hypokalemia during hypoglycemia and recommended incorporation of  $\beta$ -blockers for diabetes mellitus to reduce dangers associated with severe hypoglycemia.<sup>45</sup>

### CONCLUSIONS

In conclusion, besides prolonged cardiac repolarization, LQT2 patients display exaggerated incretin and endocrine pancreatic function with increased GLP-1, GIP, and insulin secretion and defective glucagon levels, causing lower plasma glucose levels and thus an increased risk of hypoglycemia. Pharmacological Kv11.1 blockade (dofetilide) in rats had similar effects and inhibition of *hERG* in  $\beta$  and L cells directly increased insulin and GLP-1 secretion by up to 50%. Because both postprandial hypo- and hyperglycemia influenced the QT interval in LQT2 patients, the risk of cardiac events may be further increased during glycemic excursions in LQT2. It may therefore be relevant to advise LQT2 patients to avoid



high sugar intake, which would limit both postprandial hyper- and hypoglycemia, to reduce this risk.

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## DISCLOSURES

None.

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## FOOTNOTES

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## Patients With Long-QT Syndrome Caused by Impaired *hERG*-Encoded K<sub>v</sub>11.1 Potassium Channel Have Exaggerated Endocrine Pancreatic and Incretin Function Associated With Reactive Hypoglycemia

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## **SUPPLEMENTAL MATERIAL**

### **Patients with Long QT syndrome Due to Impaired *hERG*-encoded Kv11.1 Potassium Channel Have Exaggerated Endocrine Pancreatic and Incretin Function Associated with Reactive Hypoglycemia**

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## **Supplementary Methods**

### **Oral glucose tolerance test (OGTT) in rats**

Study approval was obtained from the Danish Animal Experiments Inspectorate (2013-15-2934-00833). Female Wistar rats (~ 230 g) were purchased from Janvier Labs (Saint-Berthevin Cedex, France ) and were allowed to acclimate for at least a week in a 12:12h light-dark cycle with ad libitum access to drinking water and standard chow. Rats were handled daily the week leading up to the experiment to minimize stress. On the day of experimentation, rats were fasted from 07:30 and the experiments were performed around 17:00. Dofetilide (Tocris, Bioscience, Bristol, UK) was dissolved in PEG400/saline (1:1) on the day of experimentation. Rats were divided into weight-matched groups and received an I.V. injection under isoflurane anesthesia (4% in 100% O<sub>2</sub>) of dofetilide (5 mg/kg dofetilide) or vehicle 15 minutes prior to glucose gavage feeding. Half-life of dofetilide in female rats is 1.2 hrs<sup>1</sup>. During gavage, all rats received a bolus of 2 g/kg glucose (500 mg/ml). The moment of glucose gavage was considered time 0 min. Blood was collected at time -15 (just prior to I.V. injection), 0 (just prior to glucose gavage), 15, 30 and 60 min. The blood samples were collected by sublingual vein puncture into pre-chilled EDTA-coated capillary tubes (Microvette 200 K3E, Sarstedt, Nümbrecht, Germany) and kept on ice. Blood glucose was measured immediately after collection with an Accu-chek Compact Plus meter (Roche, Mannheim, Germany). The samples were then centrifuged (1100 g, 15 min, 4 °C) and plasma was transferred to Eppendorf tubes and instantly frozen on dry ice. Plasma was stored at -20 °C until analysis was performed. Plasma insulin levels were analyzed using rat insulin-ELISA kit (Mercodia, Uppsala, Sweden). Data were analyzed by t-test.

## Cells studies

### *Cell culture*

GLUTag cells were grown in low glucose (1.0g/l) DMEM glucose supplemented with 10 % FBS and 1% (v/v) P/S and glutamax (Cat. No. 35050061, Gibco, Life Technologies Corporation, CA, USA). Min-6 cells were kindly provided by Professor Jens Højris Nielsen (University of Copenhagen, Denmark) and grown in DMEM (Cat. No. 31966-021, Gibco, Grand Island, USA) containing 5000U/ml Pen-Strep (Cat. No. 15140-122, Gibco) and 10 % fetal bovine serum (FBS) (Cat. No. Sv3016003, Thermo, Roskilde, Denmark). The cells were seeded in 24-well plates (Nunc<sup>TM</sup>, Thermo Scientific) at a cell density of  $4 \times 10^4$  per well.

### *Quantitative polymerase chain reaction (qPCR) and small interfering RNAs*

Total RNA was extracted and isolated from cell lines and qPCR was performed with mouse *HERG*, primers (GeneCopoeia, Inc. Rockville, MD 20850 USA). The technique has previously been described<sup>2,3</sup>. Small interfering RNAs (siRNAs) against *hERG* were obtained as SMARTpool reagents from Santa Cruz Biotechnology, INC (Dallas, TX 75220 USA), and siRNA universal negative control was from Sigma-Aldrich (Brøndby, Denmark). siRNA transfection was performed using OPTI-MEM® I and Lipofectamine<sup>TM</sup> 2000 (Invitrogen). The cultured cells were transiently transfected with the plasmids or with vector control using X-treme gene® 6 Transfection Reagent (Cat. No. 063365787001, Roche Applied Science, Indianapolis, IN 46250, USA) according to the manufacturer's instructions. Relative mRNA was calculated using the  $\Delta\Delta C_q$  method<sup>4</sup>.

### *Stimulation protocol*

For acute stimulation protocols of cultured L- (GLUTag) and  $\beta$ - (Min-6) cells (with/without siRNA *hERG*), we used ~80% confluent cells from different batch numbers (n=3). Cells were

incubated for 2 h with PBS (control) or 180 mg/dl (10 mmol/l) glucose. After the end of the stimulation period, cell media were obtained and centrifuged (1,500 x g, 4°C, 5min) to remove any cells or debris and kept at -80°C until analysis.

### *Biochemical measurements*

We diluted the cell media in assay buffer from each of the respective ELISAs to obtain levels within the dynamic range of the standard curves. Levels of insulin were measured using an ELISA from Mercodia (Cat. No. 10-1249-01, Uppsala, Sweden). Levels of GLP-1 were measured using a sandwich ELISA targeting total GLP-1 (amidated and glycine-extended forms) as previously described<sup>5</sup>. One-way ANOVA with a post hoc (Sidak Holm) correction for multiple testing was used to test for significance.

## **Supplementary results**

### **Continuous glucose monitoring**

Six of the eleven LQT2 patients and six matched control subjects were examined with Continuous Glucose Monitoring (CGM) for the recommended minimum of three days<sup>6</sup>( $4.3 \pm 1.0$  days vs.  $4.3 \pm 1.0$  days,  $p = 0.9$ ). Unfortunately, none of the two patients who developed serious hypoglycemia (p-glucose <50mg/dl) during the OGTT, completed the CGM. Four out of 7 LQT2 patients (11 in total) and 2 out of the 8 controls (22 in total) with hypoglycemia (p-glucose <70 mg/dl) during OGTT also participated in the CGM.

There were no differences in 24-hour blood glucose profiles. None of the examined participants were seriously hypoglycemic (p-glucose <50mg/dl) during the continuous examination period (supplementary table 1).

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**Supplementary table 1. Continuous glucose monitoring**

<b>Glucose (mg/dl)</b>	<b>LQT2 patients</b>	<b>Control participants</b>	<b>p-value (LQT2 vs Control)</b>
>141	8 ± 4 min.	14 ± 11 min.	0.6
70-141	1408 ± 9 min.	1371 ± 17 min.	0.1
50-70	24 ± 11 min.	55 ± 19 min.	0.2
<50	0 ± 0 min.	0 ± 0 min.	1.0

**Supplementary table 1.** Continuous glucose monitoring (CGM) of 6 LQT2 patients and 6 matched control participants presented as minutes per 24-hours in each glycemic interval. Difference between cases and control participants was analyzed with a mixed model ANOVA. The two patients who developed serious hypoglycemia during the OGTT, did not complete CGM. Data are shown as mean ± SD.

**Supplementary Table 2. Delta changes in ECG parameters**

<b>Delta changes in ECG</b>	<b>LQT2 patients</b>	<b>Control participants</b>	<b>p-value</b>
Maximum Δ increase QTcB <sub>0-30 min.</sub>	25.5 ± 3.6	14.4 ± 2.7	<b>0.01</b>
Maximum Δ increase QTcF <sub>0-30 min.</sub>	19.1 ± 2.6	8.6 ± 1.9	<b>0.004</b>
Maximum Δ increase HR <sub>0-30 min.</sub>	5.2 ± 1.6	5.9 ± 1.3	0.6

**Supplementary Table 2.** Delta changes in ECG parameters in 11 *hERG* patients and 22 matched control participants during OGTT. Acute response to glucose ingestion (0-30 min) of QTcB, QTcF and heart rate (HR) analyzed with a mixed model ANOVA. Data are shown as mean ± SD.



**Supplementary table 3. Hypoglycemia questionnaire.**

Symptom (N)	Frequency (Point)		Severity (Point)	
	LQT2 (11)	Control (22)	LQT2 (11)	Control (22)
<b>1. Craving for sweets.</b>	<b>1.5 ± 0.2*</b>	<b>2.3 ± 0.2*</b>	1.4 ± 0.2	1.5 ± 0.1
<b>2. Irritability when a meal is missed out.</b>	1.8 ± 0.4	1.4 ± 0.3	1.4 ± 0.2	1.1 ± 0.2
<b>3. Feeling tired or weak when a meal is missed out.</b>	<b>2.0 ± 0.3*</b>	<b>1.2 ± 0.2*</b>	<b>1.4 ± 0.2*</b>	<b>0.8 ± 0.1*</b>
<b>4. Often hungry.</b>	1.9 ± 0.3	2.2 ± 0.2	1.6 ± 0.2	1.6 ± 0.2
<b>5. Dizziness when suddenly standing up.</b>	<b>2.0 ± 0.3*</b>	<b>1.0 ± 0.2*</b>	1.5 ± 0.2	0.9 ± 0.1
<b>6. Feeling dizzy in general.</b>	0.7 ± 0.2	0.3 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
<b>7. Feeling tired or uncomfortable some hours after a meal.</b>	<b>1.3 ± 0.3*</b>	<b>0.6 ± 0.1*</b>	1.1 ± 0.2	0.7 ± 0.1
<b>8. Trouble concentrating.</b>	1.5 ± 0.2	1.1 ± 0.2	0.8 ± 0.2	1.0 ± 0.1
<b>9. Heart palpitations.</b>	<b>1.3 ± 0.3*</b>	<b>0.6 ± 0.1*</b>	1.1 ± 0.2	0.8 ± 0.1
<b>10. Occasional shakiness.</b>	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.1
<b>11. Occasional blurry vision.</b>	0.8 ± 0.3	0.7 ± 0.2	0.6 ± 0.2	0.7 ± 0.2
<b>12. Depression or mood swings.</b>	1.3 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.2
<b>13. Frequently anxious or nervous.</b>	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.6 ± 0.1
<b>14. Frequently aggressive.</b>	0.5 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	0.8 ± 0.2
<b>15. Frequently awakening at night.</b>	1.9 ± 0.3	1.9 ± 0.2	1.1 ± 0.2	1.4 ± 0.2
<b>16. Night sweats.</b>	1.7 ± 0.3	1.5 ± 0.2	1.3 ± 0.2	1.3 ± 1.2
<b>17. Frequently sweating.</b>	1.6 ± 0.2	1.6 ± 0.2	1.2 ± 0.2	1.1 ± 0.2
<b>18. Occasionally fainting.</b>	<b>0.6 ± 0.2*</b>	<b>0.1 ± 0.1*</b>	0.9 ± 0.4	0.4 ± 0.1

**Supplementary table 3.** Hypoglycemia questionnaire. \*Significant (p<0.05) difference

between cases and control participants performed with paired t-test. 73% (8/11) LQT2 patients had a frequency score of 25 or higher vs 27% (6/22) controls; 6 of those 8 LQT2

patients vs 2 of those 6 controls had hypoglycemic plasma glucose levels during the OGTT (<70mg/dl). 82% (9/11) LQT2 patients vs 50% (11/22) controls had a frequency score of 19 or higher; 7 (as in all LQT2 with hypoglycemia) of those 9 LQT2 patients vs 5 of those 11 control participants had hypoglycemic glucose levels (<70mg/dl) during the OGTT. 7/11 LQT2 vs 7/22 controls had a severity score of 20 or above; 6 of those 7 LQT2 and 2 of those 7 controls had hypoglycemia during the OGTT.